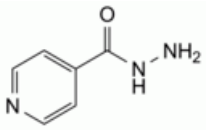
Edition: BP 2025 (Ph. Eur. 11.6 update)

Isoniazid

General Notices

(Ph. Eur. monograph 0146)



$C_6H_7N_3O$ 137.1 *54-85-3*

Action and use

Antituberculosis drug.

Preparations

Isoniazid Injection

Isoniazid Oral Solution

Isoniazid Tablets

Ph Eur

DEFINITION

Pyridine-4-carbohydrazide.

Content

99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline powder or colourless crystals.

Solubility

Freely soluble in water, sparingly soluble in ethanol (96 per cent), practically insoluble in heptane.

IDENTIFICATION

First identification: A, B.

Second identification: A, C.

A. Melting point (2.2.14): 170 °C to 174 °C.
B. Infrared absorption spectrophotometry (2.2.24).

Comparison *isoniazid CRS*.

C. Dissolve 0.1 g in 2 mL of *water R* and add 10 mL of a warm 10 g/L solution of *vanillin R*. Allow to stand and scratch the wall of the test tube with a glass rod. A yellow precipitate is formed. Recrystallise from 5 mL of *ethanol (70 per cent V/V) R*. The crystals, dried at 100-105 °C, melt (2.2.14) at 226 °C to 231 °C.

TESTS

Solution S

Dissolve 2.5 g in *carbon dioxide-free water R* and dilute to 50 mL with the same solvent.

Appearance of solution

Solution S is clear (2.2.1) and not more intensely coloured than reference solution BY_7 (2.2.2, *Method II*).

pH (2.2.3)

6.0 to 8.0 for solution S.

Impurity E

Liquid chromatography (2.2.29). *Use freshly prepared solutions*.

Solvent mixture *acetonitrile R*, *water R* (50:50 *V/V*).

Solution A Dilute 1 mL of *benzaldehyde R* to 50 mL with *methanol R*. Use the solution within 4 h.

Test solution Dissolve 50.0 mg of the substance to be examined in 1 mL of *water R* and mix with 5 mL of solution A. Shake and allow to stand for 45 min to allow completion of the derivatisation reaction (hydrazine (impurity E) reacts with benzaldehyde to give benzaldehyde azine). Dilute to 10.0 mL with the solvent mixture.

Reference solution Dissolve 20.0 mg of *hydrazine sulfate R* (equivalent to 4.925 mg of hydrazine (impurity E)) in *water R* and dilute to 50.0 mL with the same solvent. Dilute 2.5 mL of the solution to 100.0 mL with *water R*. Mix 1.0 mL of this solution and 2.5 mL of solution A. Shake and allow to stand for 45 min to allow completion of the derivatisation reaction (hydrazine (impurity E) reacts with benzaldehyde to give benzaldehyde azine). Dilute to 25.0 mL with the solvent mixture. Dilute 7.5 mL of this solution to 10.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, Ø = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase *water for chromatography R*, *acetonitrile R* (40:60 *V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 300 nm.

Injection 10 µL.

Run time 1.5 times the retention time of benzaldehyde azine.

Retention time Benzaldehyde azine = about 14 min.

System suitability Reference solution:

— *repeatability*: maximum relative standard deviation of 5.0 per cent for the area of the peak due to benzaldehyde azine determined on 6 injections.

Calculation of content:

— for impurity E, use the concentration of hydrazine in the reference solution and the peak areas due to benzaldehyde azine.

Limit:

— *impurity E*: maximum 15 ppm.

Related substances

Liquid chromatography (2.2.29). *Use freshly prepared solutions*.

Buffer solution Dissolve 13.6 g of potassium dihydrogen phosphate R in 950 mL of water for chromatography R, adjust to pH 6.9 with strong sodium hydroxide solution R, add 30 mg of triethanolamine R and dilute to 1000 mL with water for chromatography R.

Test solution Dissolve 25.0 mg of the substance to be examined in mobile phase A and dilute to 25.0 mL with mobile phase A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with mobile phase A.

Reference solution (b) Dissolve 5 mg of isonicotinic acid R (impurity A), 5 mg of isonicotinamide R (impurity B) and 5 mg of nicotinoyl hydrazide R (impurity D) in mobile phase A and dilute to 50 mL with mobile phase A. Dilute 1 mL of the solution to 10 mL with mobile phase A. Dilute 1 mL of the solution obtained to 10 mL with the test solution.

Column:

— *size*: *l* = 0.25 m, *Ø* = 4.6 mm;

— *stationary phase*: base-deactivated end-capped octadecylsilyl silica gel for chromatography R (5 µm).

Mobile phase:

— *mobile phase A*: methanol R, buffer solution (3:97 *V/V*);

— *mobile phase B*: methanol R;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 12	100	0
12 - 20	100 → 85	0 → 15
20 - 28	85	15

Flow rate 1.5 mL/min.

Detection Spectrophotometer at 266 nm.

Injection 10 µL.

Identification of impurities Use the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, B and D.

Relative retention With reference to isoniazid (retention time = about 9 min): impurity A = about 0.4; impurity D = about 1.15; impurity B = about 1.4.

System suitability Reference solution (b):

— *peak-to-valley ratio*: minimum 1.8, where H_p = height above the baseline of the peak due to impurity D and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to isoniazid.

Calculation of percentage contents:

— *correction factors*: multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 1.4; impurity B = 1.5;

— for each impurity, use the concentration of isoniazid in reference solution (a).

Limits:

— *impurities A, B*: for each impurity, maximum 0.15 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *total*: maximum 0.5 per cent;

— *reporting threshold*: 0.05 per cent.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

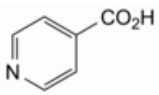
Dissolve 0.250 g in *water R* and dilute to 100.0 mL with the same solvent. To 20.0 mL of the solution add 100 mL of *water R*, 20 mL of *hydrochloric acid R*, 0.2 g of *potassium bromide R* and 0.05 mL of *methyl red solution R*. Titrate dropwise with *0.0167 M potassium bromate*, shaking continuously, until the red colour disappears.

1 mL of *0.0167 M potassium bromate* is equivalent to 3.429 mg of $C_6H_7N_3O$.

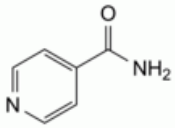
IMPURITIES

Specified impurities *A, B, E.*

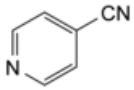
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *C, D.*



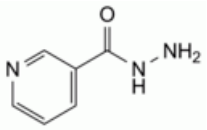
A. pyridine-4-carboxylic acid (isonicotinic acid),



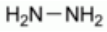
B. pyridine-4-carboxamide (isonicotinamide),



C. pyridine-4-carbonitrile (isonicotinonitrile),



D. pyridine-3-carbohydrazide (nicotinoyl hydrazide),



E. hydrazine.

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